NATIVE PARASITE COMBATING AN INVASIVE SPECIES:

AN OOMYCETE VS. *ECHINOGAMMARUS ISCHNUS*

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NATIVE PARASITE COMBATING AN INVASIVE SPECIES:

AN OOMYCETE VS. *ECHINOGAMMARUS ISCHNUS*

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SUMMARY

In the context of invasions in ecology, parasites can play an important role in mediating the outcomes of competition between the native and invasive species. For example, a native parasite in the upper St. Lawrence River area has been found infecting both native and non-native gammarid amphipods, *Gammarus fasciatus* and *Echinogammarus ischnus,* respectively. Usually when the non-native species invades an area, the native amphipod is rapidly replaced by the non-native species. However, in this specific region the native amphipod was not replaced by the non-native species, and the non-native species was observed to be infected by a parasite. To determine infection rates in the native and non-native hosts, DNA was extracted from both species of amphipods and specific primers for the 18S rRNA gene were created to generate quantitative analysis of the incidence of the parasite in the amphipods. These data indicate that the effect of the parasite on the native and non-native amphipods could be the mechanism preventing the dominance of the non-native species in this specific area for the past ten years, since the parasite was found to infect the non-native amphipod more frequently and at a higher level than the native amphipod.

INTRODUCTION

A worldwide invasion has begun: non-native animals are invading new environments and driving native populations to local extinction(Berezina, 2007). Freshwater environments, especially, are being invaded at exponential rates (Cornet, Sorci, & Moret, 2010). Some invasions can have very little negative impact; however, others can have tremendous effects and lead to dramatic changes in biodiversity (Hogg, de Lafontaine, & Eadie, 2000). One way of predicting the success of the non-native and native species, which has been relatively undocumented, involves investigating their vulnerability to native or non-native parasites (Torchin et. al, 2002). In many instances where the non-native species takes over, the native species proves vulnerable to the new parasites that the non-native species introduces to the environment. However, in certain cases parasites have been able to delay an invasion (Prenter et al., 2003). Current research focuses mainly on studying non-native species that have successfully invaded a new habitat. This bias towards non-native species skews the ability of investigators to observe the effect of native parasites on non-native species. Overlooked failed invasions may offer more insight into the role of native parasites than the study of only the successful invasions.

Common Invasion Dynamics

Coexistence

As a non-native species invades a certain area, competition occurs between the native and non-native species that occupy the same niche and require the same resources.

In some instances, both the native and non-native species are able to coexist(MacNeil, 2001). Thus, the ability to coexist indicates that the parasites of both species do not present a substantial negative impact on the other (MacNeil, 2001).

Successful Invasion

The more commonly documented occurrence, upon invasion of an environment, is for the non-native species to overtake the native species. In cases where the two species are taxonomically and trophically similar, it is often assumed that interspecific competition is the only type of interaction occurring between the species resulting during an invasion (Dick, 2008). However, competition is not the only studied mechanism; predation is also commonly studied mechanism regarding invasion dynamics of a system. In some instances the new species drives the native species to extinction via predation. For example, this type of extinction occurs with some amphipods due to the invasive species being much larger than their native counterparts (Berezina, 2007).

Although predation and competition have been the predominantly studied mechanisms regarding the impact of invasive species, many new studies are focusing on the impact of parasites (Kelly, Paterson, Townsend, Poulin, & Tompkins, 2009). Recent work suggests that successful invasions occur due to the high level of genetic polymorphism in most non-native species (Berezina, 2007). High levels of genetic polymorphism suggest that within a certain area, a non-native species can have a large range of both sizes and structures. Having such a wide range of features can give the non-native species a competitive advantage over the native species. For example, some of the forms of the non-native species could be more resistant to parasites (Berezina, 2007).

Older studies investigated the influence of parasites on the success of an invasion through intraguild predation, which can be controlled by biotic (parasites) and abiotic (water chemistry) factors (Dick, 2008). Parasitism can weaken a population, making it more susceptible to attack. For example, in cases where parasites selectively infect one species over another, a higher rate of predation can often be observed in the species that has been weakened by infection (Dunn & Dick, 1998). In addition, water chemistry, specifically in regards to decreased oxygen levels, can also stress a population, making it more vulnerable to predation..

Parasite-mediated apparent competition has also played a major role in the invasion success of non-native species (Holdich, 1991; Settle & Wilson, 1990). This type of apparent competition occurs when an additional host (such as the invasive species) is introduced to a system and alters the equilibrium of a food limited parasite. The additional host can increase a parasite's rate of infection on a different host species in the same area. Such dynamics have mainly been attributed to non-native parasites that are introduced alongside the invading non-native species (Dunn & Dick, 1998). However, since parasitism can mediate the competition between hosts and thereby ultimately affect the success of a biological invasion (Kohler, 1997) it would be of interest to study whether a native parasite can play a similar role in apparent competition.

Two major theories in this area of competition are the ideas of parasite spillback and spillover (Kelly, et al., 2009). Parasite spillback suggests that the native parasites are taken up by the non-native species and are then returned to the native species. The "spilled back" parasite proves to be stronger and more abundant, causing the death of the native species (Kelly, et al., 2009). However, in parasite spillover, the non-native

asymptomatic species enters the new system bearing a non-native parasite that is fatal to the native species (Prenter, MacNeil, Dick, & Dunn, 2004). The non-native species acts as a reservoir for the parasite resulting in a "spillover" to the native species (Prenter, et al., 2004). Ultimately, parasite spillback supports the idea that non-native species may be better able to withstand the infection of the native parasite. These conclusions, however, are in direct opposition to some new data concerning the dynamics involved with native parasites infecting non-native species (Kestrup, et al., 2011).

Failed Invasion

Non-native species possess many adaptations that should make them a threat towards the native species; however, when the non-native species does not successfully invade an environment some other mechanism must be acting on the system. Unfortunately, there has not been a large sum of empirical evidence gathered regarding the mechanisms of competition between the native and non-native species involved in a failed invasion (Dick, 2008). However, there is evidence from a recent study by Kestrup and Ricciardi that the native parasites may be infecting the non-native species at a greater rate than the native species.

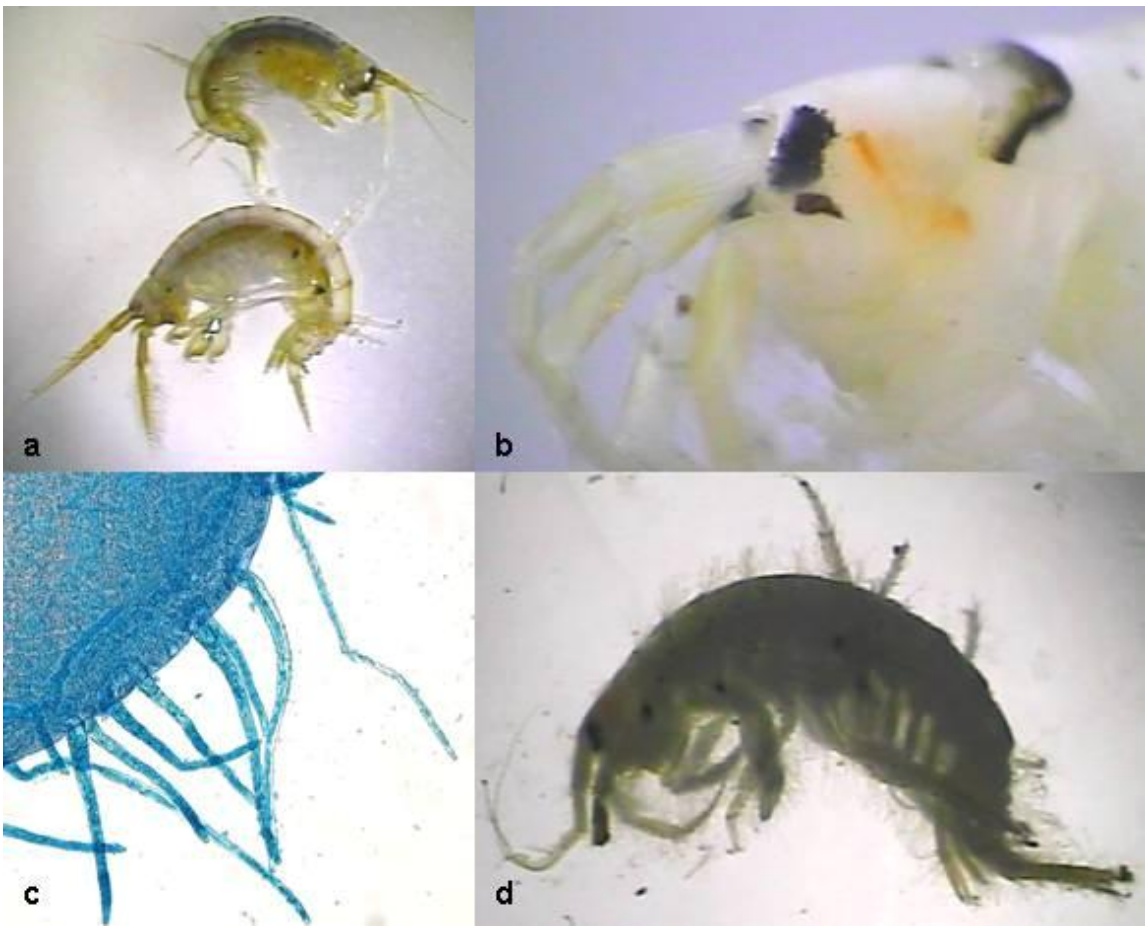
System of Study

An ideal system for the study of a "failed invasion" is that of the upper St. Lawrence River amphipods. Amphipods are shrimp-like crustaceans that can live in the water column or on the lake bottom. The two species from the St. Lawrence, the native *Gammarus fasciatus* and the non-native *Echinogammarus ischnu*s, are of interest because

the non-native species has yet to dominate the region. In all other regions the non-native species has taken over the environment of the native species (MacNeil, 2001).

The system studied by Kestrup and Ricciardi was in the upper St. Lawrence River region. They found that the non-native amphipods were being infected by a parasite (Figure 1). The native amphipod was also being infected; however, it was able to live while infected whereas the non-native amphipod died when infected. However, they could not identify the parasite morphologically, nor could they quantify the levels of infection.

Figure 1: Infected live female (top) and male (bottom) *E. ischnus* with melanized spots (a), newly dead *E. ischnus* (b), hyphae growing out from a gill (c), and a dead *E. ischnus* overgrown with hyphae(d). (Photos taken by Åsa Kestrup)



In order to properly identify the parasite and the infection, it is necessary to use molecular techniques to extract and analyze the DNA of the parasite in the amphipods. In this study, we analyzed the DNA from both infected and uninfected individuals of both

species. PCR of the DNA allowed for the quantification of infection by the native parasite in both the native and non-native amphipods.

Adding infection rate data to previous research solidifies the mechanism of a native parasite having a larger effect on a non-native amphipod instead of the opposite, more common case. It is expected that the analysis of infection by the parasite will demonstrate that the native amphipod has a greater resistance to the parasite than the non-native amphipod. Observing such a result would add a new level of understanding into the mechanisms regarding the dynamics between native parasites and non-native amphipods. Additionally, it would allow for more investigation of native parasites and the benefits that they bestow, shedding a new light on the perception of only non-native parasites having an important effect on invasion dynamics. Having a non-native amphipod be more highly infected by a native parasite supports the work of Hay et. al, regarding the "enemy of my enemy is my friend"(Hay, et al., 2004). This work explains how an organism that negatively affects a species A's "enemy" could potentially be species A's "friend" because such an enemy harms species A's enemy, thereby ultimately helping species A to succeed. Furthermore, molecular results in favor of native parasites would demonstrate that although in certain areas native amphipods are more highly infected (Dunn & Dick, 1998), in other areas non-native amphipods are most highly infected.

METHODS

DNA Extraction and Quantification

The level of infection in each of the amphipods was determined through a semi-quantitative polymerase chain reaction (PCR). A 50 ml Powersoil DNA Isolation Kit (MO BIO Laboratories, Inc.) was used to extract DNA from the individual amphipods. The power beads and liquid were decanted into a 2.0ml microcentrifuge tube. The liquid (with no power beads) was then returned to the powerbead tube, and a single preserved animal was inserted using sterile forceps. The specimen was ground using a sterile microcentrifuge pestle (Bel-Art Products, Pequannock, NJ) in the power bead tube until finely ground. The power beads were then returned to the powerbead tube containing the homogenated sample. From there, the MO BIO protocol was followed. If the DNA extraction did not immediately follow grinding of the samples, the powertubes were stored at 4°C overnight. Storing overnight has a very small effect on the amount of DNA extracted (MO BIO).

Once extracted, the DNA levels were quantified using a Nanodrop ND-1000 (Thermoscientific, Wilmington, DE). DNA concentrations were normalized to 1ng/µl. A dilution series from the normalized DNA was performed resulting in 0.1 ng/µl and 0.01ng/µl concentrations.

The semi-quantitative PCR was conducted with 3 concentrations of DNA: 5 ng/µl, 0.5ng/µl, and 0.05 ng/µl. These three concentrations resulted in 3 levels of infection intensity; High (0.05ng), Intermediate (0.5ng), and Low(5ng). For example, an infection would be classified as "high intensity" if the parasitic amplicon is detected in 0.05ng/µl of amphipod DNA. Also, the infection level revealed the amount of parasite biomass in

each amphipod. The 18S primers used in the PCR were (5'-ACCTGGTTGATCCTGCCAG-3', 5'-TGATCCTTCYGCAGGTTCAC-3'). The PCR was run according to the following recipe: 4 µl Buffer, 0.5µl dNTPs, 1.2 µl$MgCl_2$, 2 µl Primer 1, 2 µl Primer 2, 0.2 µl Taq Polymerase, and 5.1 µl dH_2O per each reaction. To each tube, 15µl of the master mix and 5 µl of template DNA was added. The agarose gel was made with 1.5 g agarose added to 100ml TAE buffer (40mM Tris Base in 20mM acetic acid, 1mM EDTA, pH 8.5). The gels were run at 90V for 60 minutes. The run gels were stained in ethidium bromide (1µg mL^{-1}) for 60 minutes. The intensity level was determined from the gels. The expected size of the parasitic amplicon was 693 base pairs in length.

Clone Library and Sequencing

Clone libraries were created in order to identify the unknown native parasite infecting the amphipods. Libraries were made for the 18S rRNA, LSU rRNA, and ITS rRNA genes. A PCR was run for each of these genes with template DNA from the amphipods. Each PCR was run with the primers specific to each gene. The PCR conditions were the same as used for the infection intensity study above.

The PCR products were cleaned using a Promega PCR clean-up kit, according to the kit's protocol. PCR products were cloned into the TOPO vector pCR 2.1 (Ivitrogen) following the manufacturer's protocol. Clones were grown on LB/SGal plates with 100µl ampicillin. 47 white colonies were chosen and run through a PCR with M13 primers. PCR products of the correct size were chosen and cleaned with the Promega kit. Using a Nanodrop, the clean PCR products were quantified.

For sequencing, a 96-well plate was set up with 2 µl primer and 200ng PCR product per well. Sequencing was performed by the Nevada Genomics Center (University of Nevada, Reno, NV).

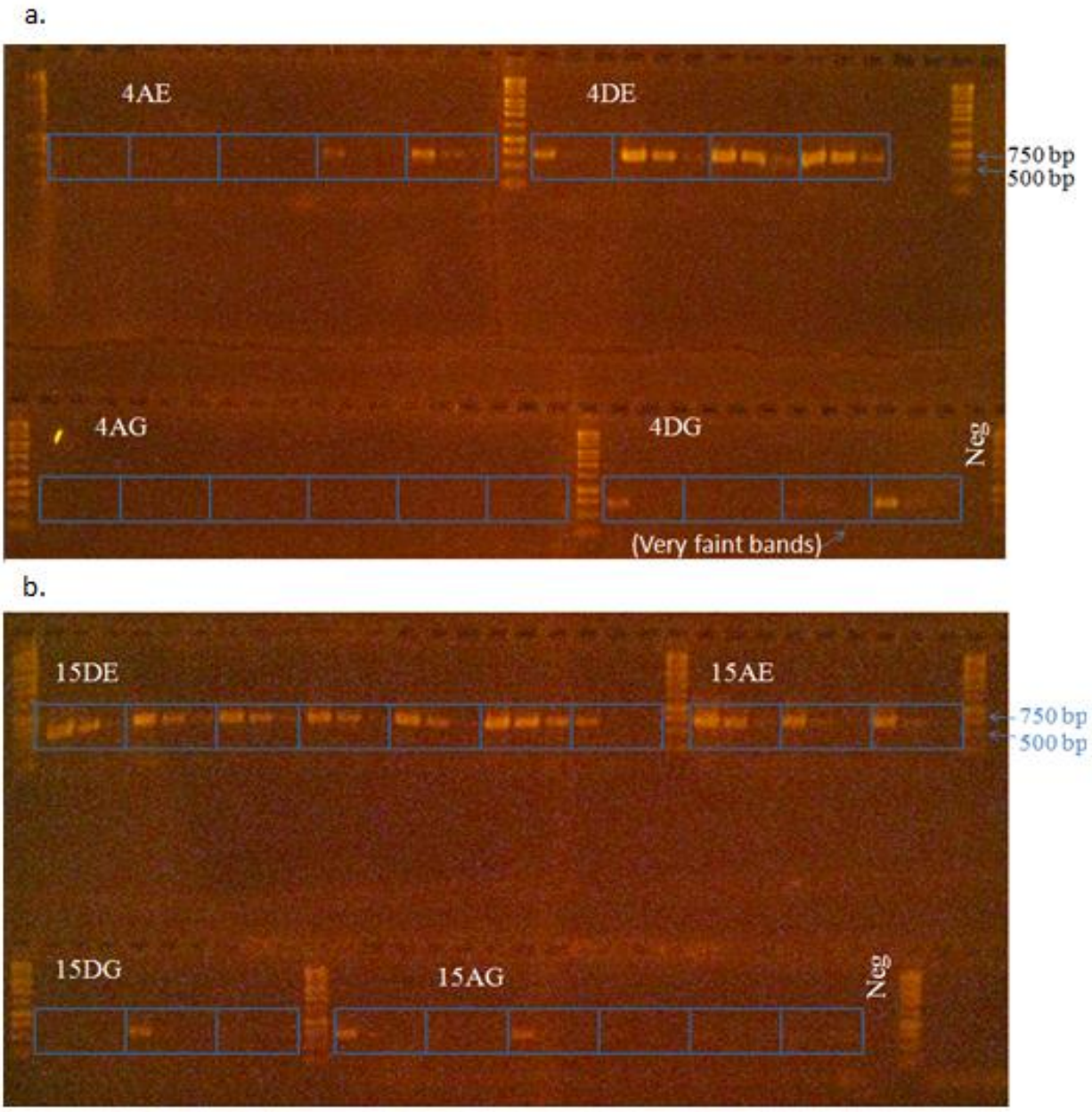
RESULTS

Parasite Levels

Parasite levels were determined by evaluating a total of 176 amphipods (89 *Echinogammarus ischnus* and 87 *Gammarus fasciatus*) in 9 sets of randomly chosen samples (Replicates 1,4,5,7,8,9,10,14,15). The infection intensities of the parasite were determined through semi-quantitative PCR. Extracted amphipod DNA was normalized to 1ng/µl and then diluted, resulting in 3 DNA concentrations for amplification. The infection intensity of the parasite in each amphipod was determined as high (0.05ng), intermediate (0.5ng), or low (5ng), depending on whether parasite DNA was detected at each different concentration. The intensities were not determined by the brightness of the band on the gel, but rather by the appearance of, or lack of a band (Figure 2a and 2b).

The amplicon for the 18SrRNA gene of the parasite was expected to be 693 base pairs in length, therefore the bands in Figure 1a and 1b can be inferred to be the amplicons of the parasite. The gels shown in Figure 1a and 1b only represent two of the amphipod samples. The gels from the other 7 samples can be found in the appendix.

Figure 2: *Saprolegnia* PCR gel for replicate 4(a) and replicate 15(b). DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length.



Of the 89 *Echinogammarus ischnus* individuals, 41 individuals died during the previous experiment (Kestrup, et al., 2011) and 48 individuals survived. The parasite was detected in 41/41 of the dead *E. ischnus* and in 10/48 of the live *E. ischnus* (Table 1). Of the 87 *Gammarus fasciatus* individuals, 23 animals died during the previous experiment (Kestrup and Ricciardi) and 64 animals were alive at the end of the experiment. Detectable amounts of parasite DNA was found in 10/23 animals of the dead *G.fasciatus* and in 7/64 of the live *G.fasciatus* (Table 1).

Table 1: Amphipods Containing Parasite DNA Above Detection Level

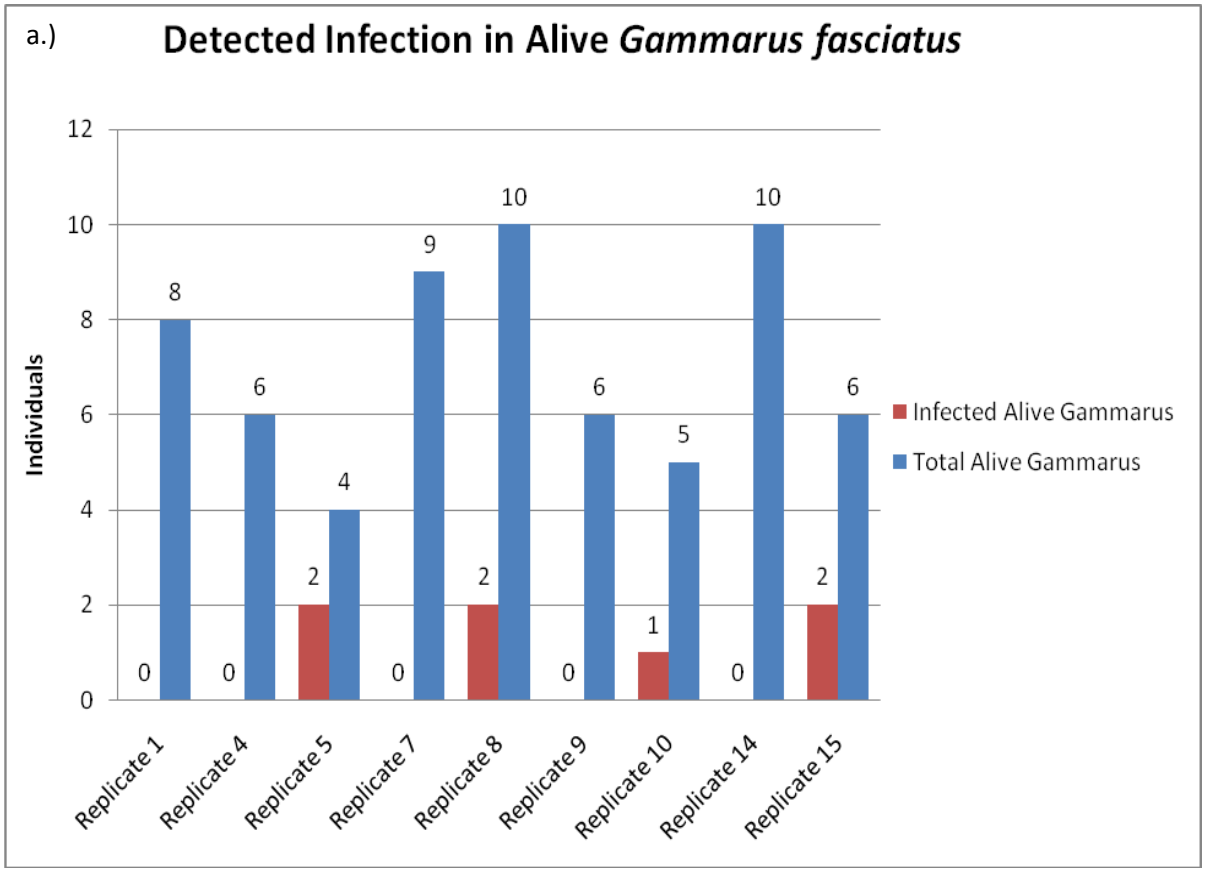
	Alive *E.ischnus*	**Dead *E.ischnus***	**Alive *G.fasciatus***	**Dead *G.fasciatus***
Replicate 1	0/6	4/4	0/8	0/0*
Replicate 4	2/5	4/4	0/6	2/4
Replicate 5	1/6	5/5	2/4	5/6
Replicate 7	2/7	2/2	0/9	1/1
Replicate 8	2/7	3/3	2/10	0/0*
Replicate 9	0/5	5/5	0/6	0/4
Replicate 10	0/5	5/5	1/5	1/5
Replicate 14	0/4	6/6	0/10	0/0*
Replicate 15	3/3	7/7	2/6	1/3
Total	10/48	41/41	7/64	10/23

*** no samples in category**

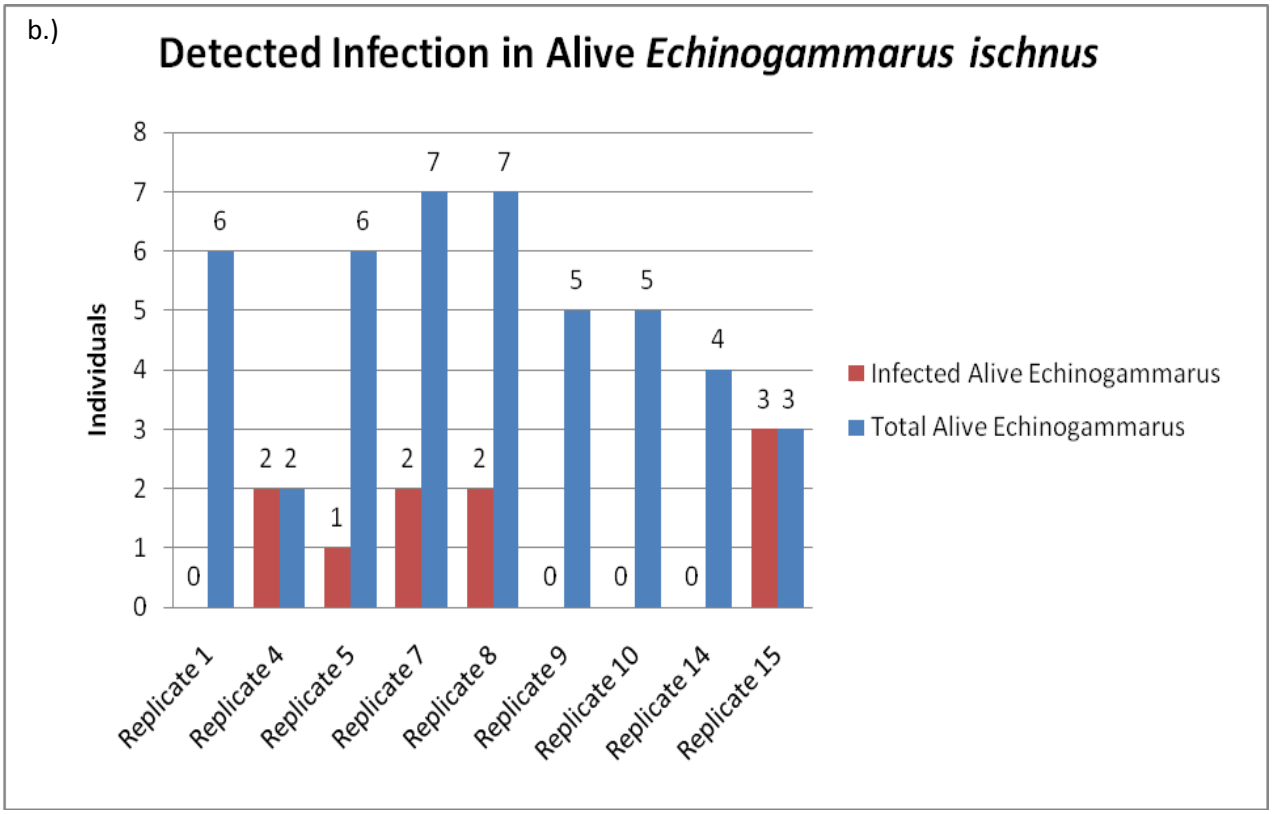
When comparing both dead and live animals of each species, the parasite was more often found in *E.ischnus* (51/89) than in *G.fasciatus* (17/87) (Fisher Exact 1-tailed, $p<0.0001$). There was no statistically significant difference between the presence of the

parasite in the live animals (Figure 3a and 3b), *E.ischnus (*10/48) and *G.fasciatus*(7/64; Fisher Exact 1-tailed, p = 0.1864).

Figure 3: Live Amphipods, (3a) *G.fasciatus* and (3b) *E.ischnus* containing parasite DNA above detection level

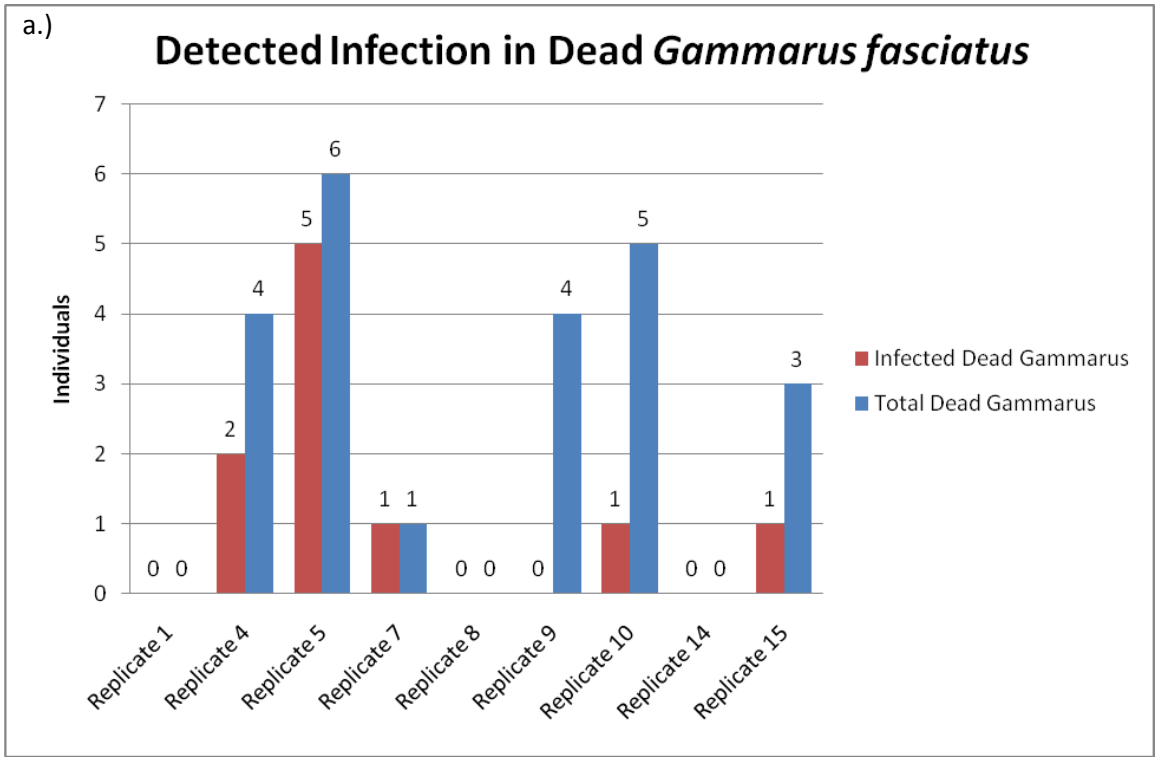


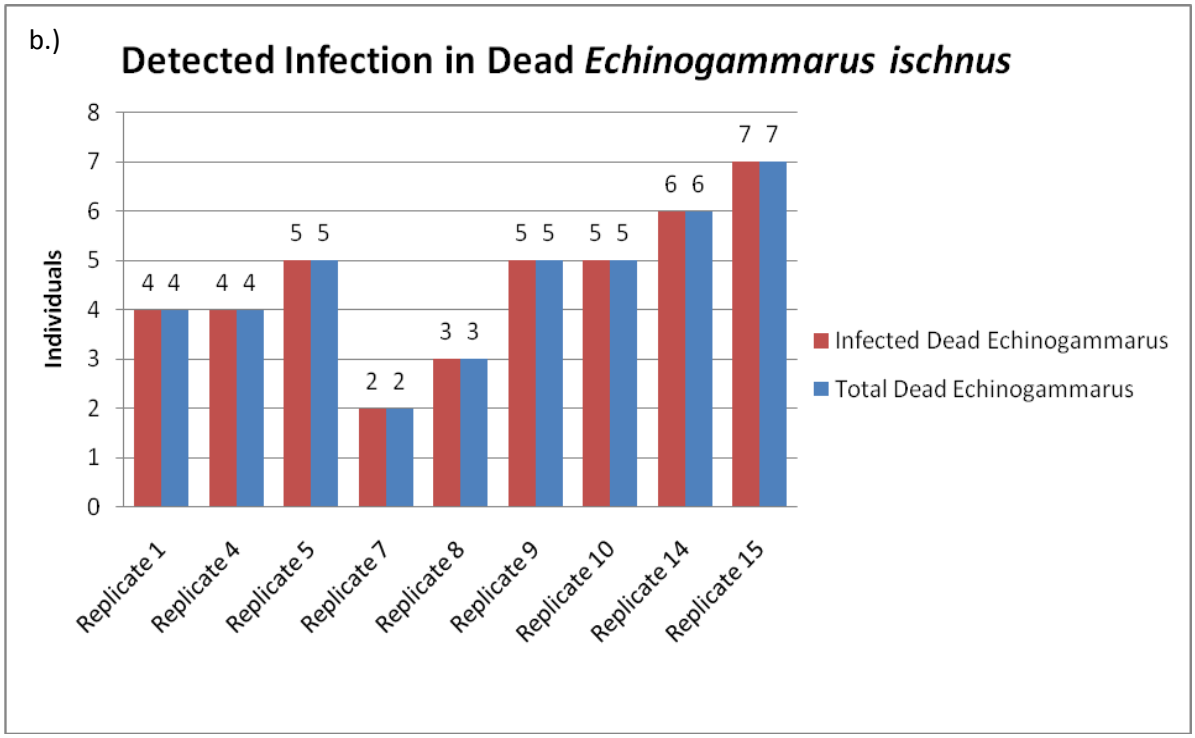
b.)



Among the dead animals (Figure 4a and 4b) *E.ischnus* (41/41) was more frequently infected with the parasite than *G.fasciatus* (10/23) (Fisher Exact 1-tailed; $p < 0.0001$).

Figure 4: Dead Amphipods, (4a) *G.fasciatus* and (4b) *E.ischnus* containing parasite DNA above detection level.





By using three DNA concentrations and by comparing the three intensity levels of the parasite within each amphipod we are essentially determining the relative amounts of parasitic biomass within the individuals. The dead *E.ischnus* contained a higher level of parasitic biomass than the dead *G.fasciatus* (Figure 5a and 5b)(Mann Whitney one-tailed; $Z=4.757$; $U=639.5$; $p<0.0001$)

Similarly, comparing the intensity levels of the parasite in the live animals gives insight into the relative biomass of the parasite in the two species. There was, however, no significant difference in the infection intensity between the live *E.ischnus* and the live *G.fasciatus* (Figure 6a and 6b ; Mann Whitney one-tailed; $Z=0.640$; $U= 401.5$; $p=0.261$)

Figure 5: Detectable parasite levels in the (5a) dead *G.fasciatus* and the (5b) dead *E.ischnus* at 3 DNA concentrations: 5 ng, 0.5 ng, and 0.05 ng.

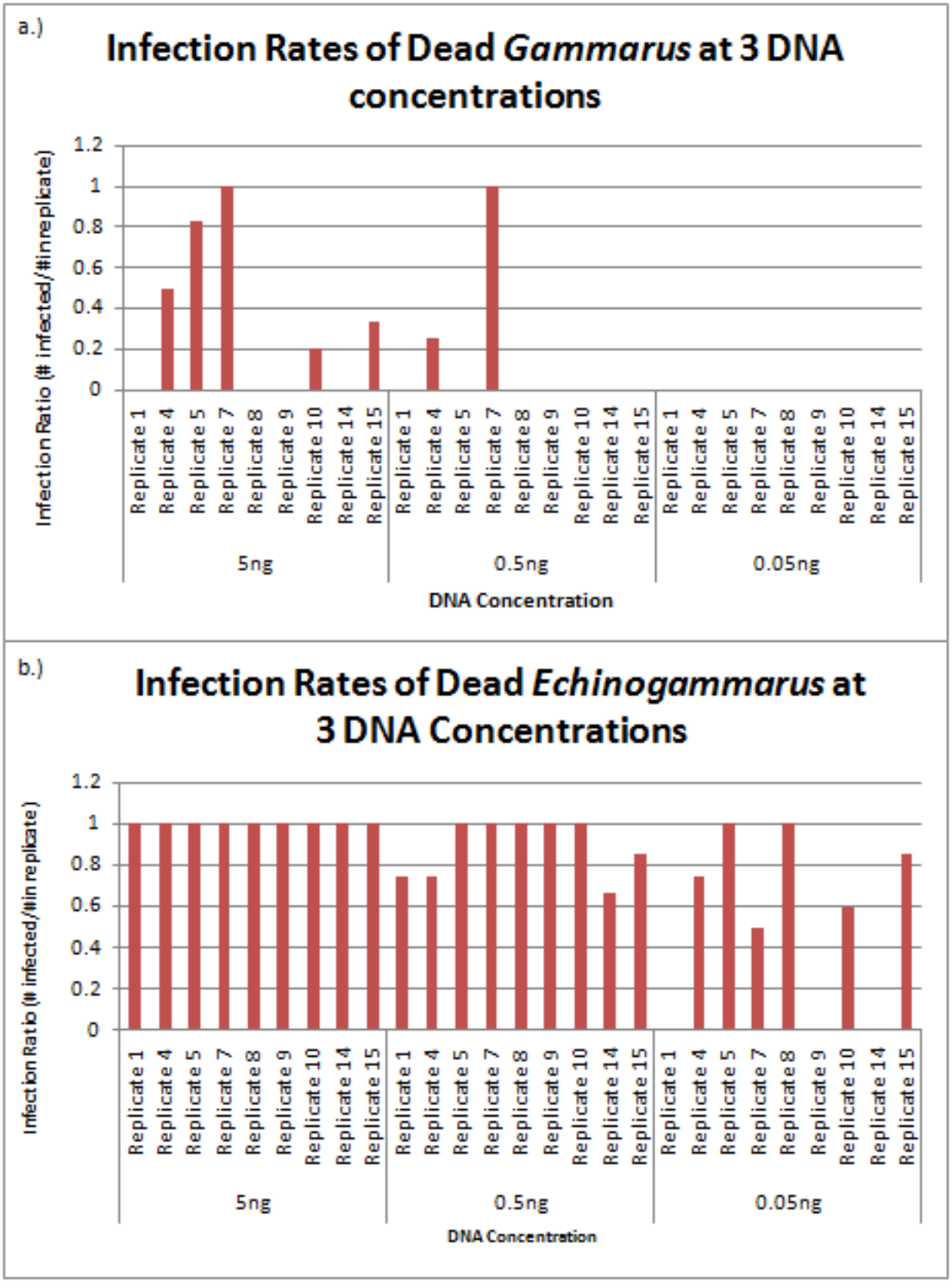
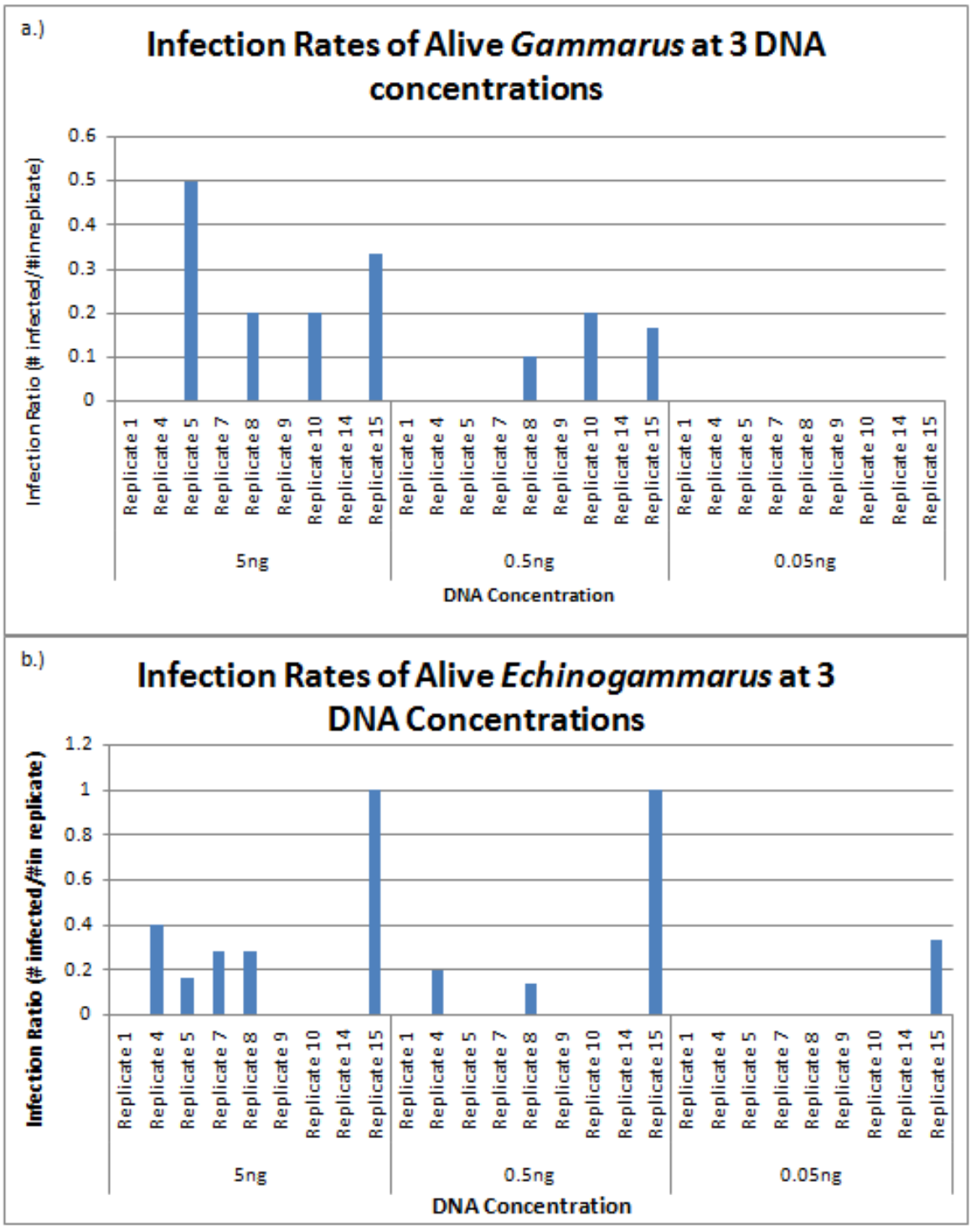


Figure 6: Detectable parasite levels in (6a) alive *G.fasciatus* and (6b) alive *E.ischnus* at 3 DNA concentrations: 5 ng, 0.5 ng, and 0.05 ng.



Parasite Identification

Sequences were obtained from 28/30 of the screened SSU rRNA clones from the dead *E.ischnus (*Figure 7a). Of these sequences, 8 clones corresponded to the sequences in the genus *Saprolegnia.* The 20 other clones corresponded to sequences of the family Gammaridae which would be due to host DNA. No other parasite sequence was found in the clone sequences. Identical sequences were obtained from the *G.fasciatus* clone libraries.

Similar results were obtained from the LSU (Figure 7b) and the ITS (Figure 7c) clone libraries of the dead *E.ischnus.* The sequences either corresponded to amphipods or to oomycetes in the Saprolegniaceae family. BLAST analysis showed that the parasites SSU rRNA sequence was 99% similar to that of *Leptolegnia caudata* (Dick et al. 1999; Figure 7a). The LSU sequence was almost identical to the *Saprolegnia* sp. WM 3 sequence (Wolinska et al. 2008; Figure 7b). The ITS sequence was also very closely related to *Saprolegnia* sp. WM 3 sequence (Figure 7c).

Figure 7: Phylogenetic trees based on (a) the SSU rRNA gene, (b) the large subunit rRNA (LSU), and (c) the rRNA internal transgenic spacer region (ITS) of the unknown oomycete parasite and several other oomycetes from the NCBI GenBank database (accession number given). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site, as indicated by the scale bar. Numbers above and below the nodes are bootstrap values for 500 replicates. Gray font indicates clone sequences of uncultured representatives.

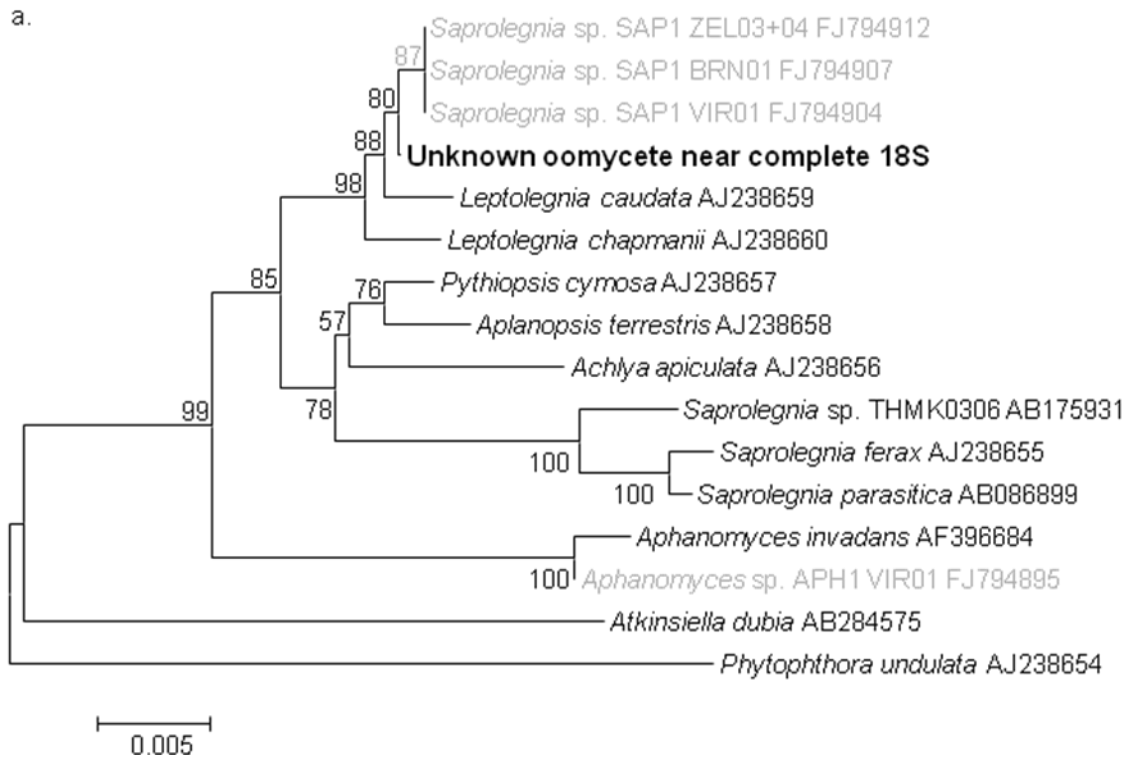


Figure 7: (cont'd)

b.

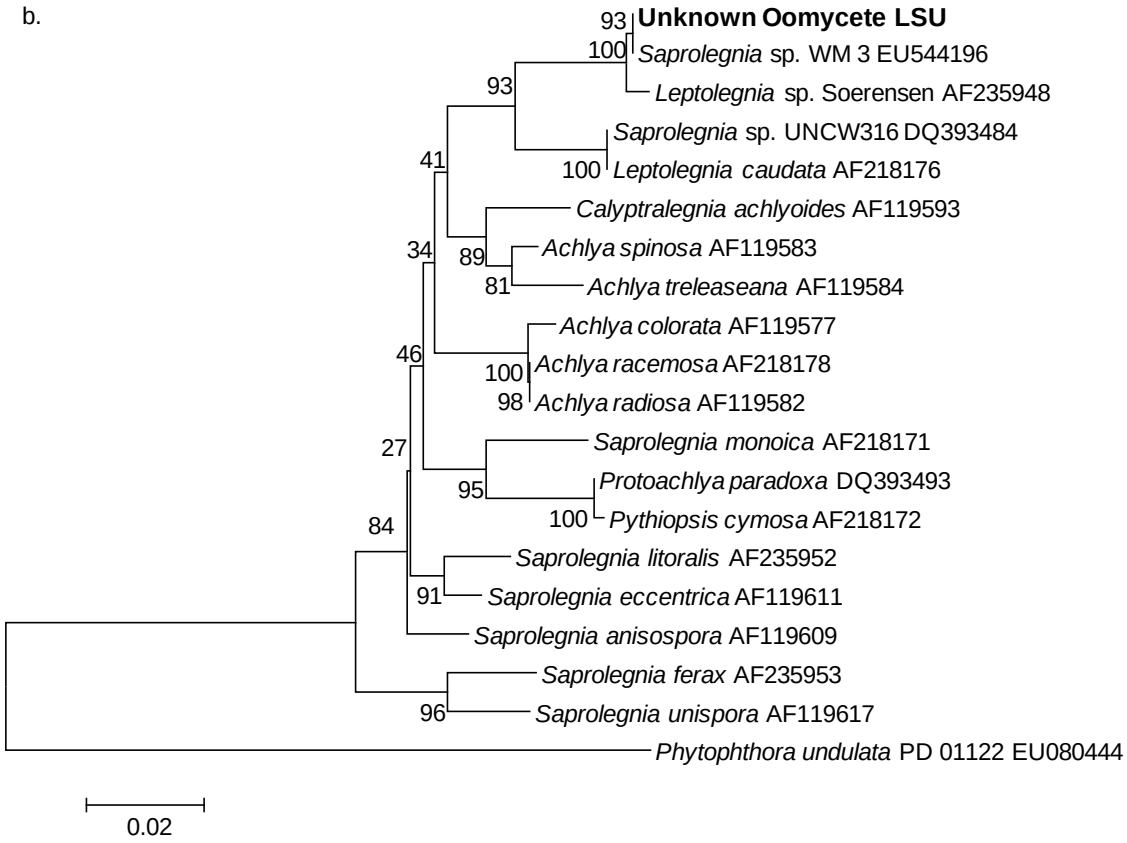
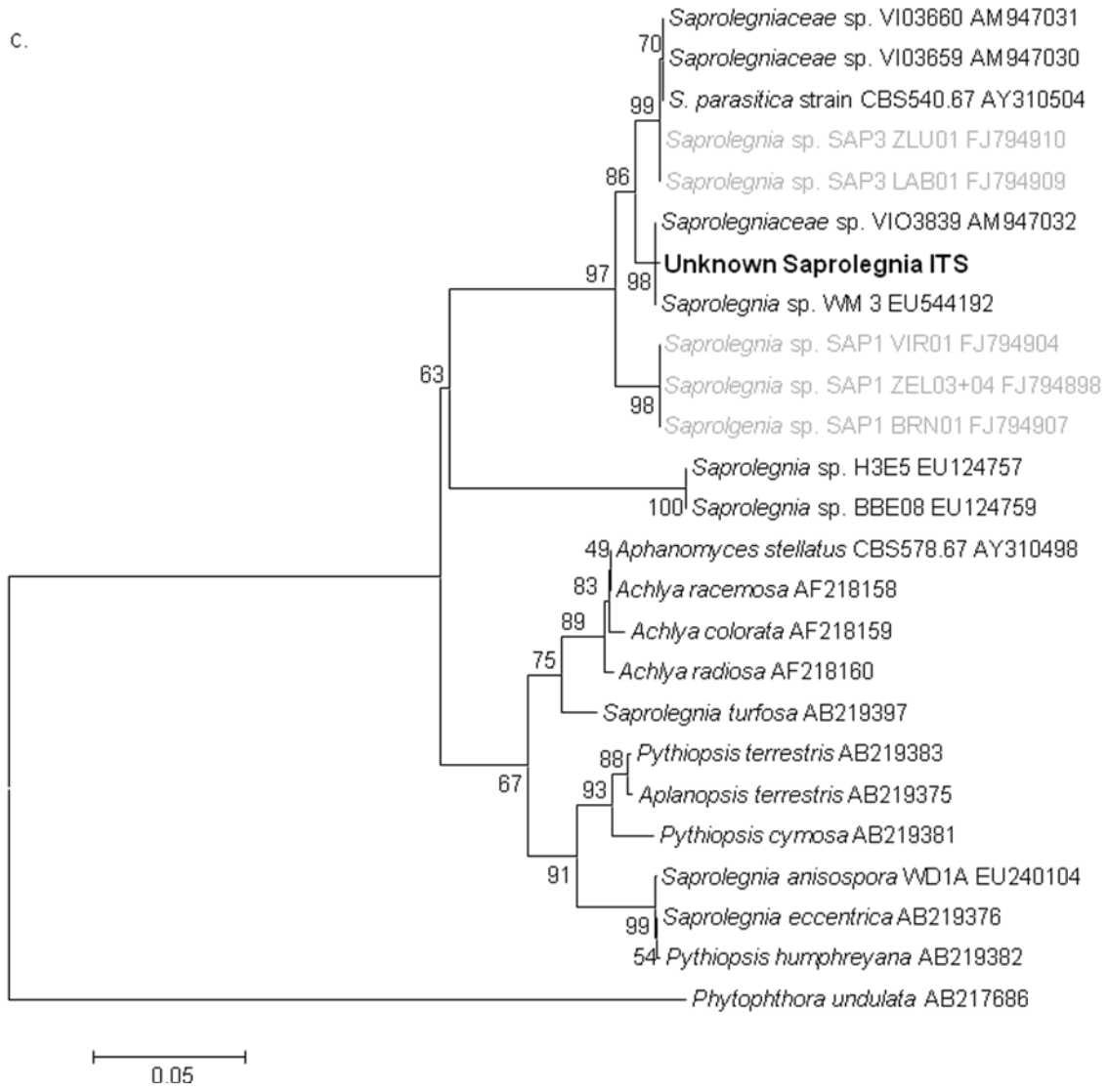


Figure 7: (cont'd)



CONCLUSION

DNA extraction and PCR quantification analysis illustrates that the parasite was more frequently detected in *E.ischnus* than in *G.fasciatus.* In comparing dead animals, *E.ischnus* (41/41) were all infected by the parasite, whereas only a small portion of *G.fasciatus* were infected (10/23). This pattern, along with the data from the infection intensities, suggests that the native *G.fasciatus* is more resistant to infection by the parasite.

There were no significant differences between the parasite levels in the live *E.ischnus* and *G.fasciatus.* Despite there not being a significant difference in the parasite levels within the live amphipods, there were still more dead *E.ischnus* (41 individuals) in comparison to *G.fasciatus* (23 dead individuals). Since both amphipods had similar levels of parasite, yet one died more frequently than the other, it could be argued that the parasite kills the invasive *E.ischnus* more quickly than the native *G.fasciatus.* This also suggests the question of whether a smaller amount of parasite can kill the *E.ischnus.*

Phylogenetic analysis of the parasite reveals that it is in the Saprolegniaceae family. This discovery is expected because many members of the Saprolegniaceae family are found to infect a variety of aquatic animals (Ramaiah, 2006). For example, this family has been found to infect insects, crayfish and fish in North America (Kiziewicz & Nalepa, 2008). An unidentified *Saprolegnia* species has been infecting an invasive crayfish, *Orconectes limosus,* in Germany (Hirsch, Nechwatal, & Fischer, 2008) Similarly to what has been observed with this current study in the St. Lawrence River

Area, the unidentified *Saprolegnia* species has led to the decline of the invasive crayfish species in Germany (Hirsch, et al., 2008).

With this experiment we did not fulfill Koch's Postulates; however, many parasite studies cannot fulfill these requirements because of the difficulty in culturing parasites(Jacomo 2002). Because of the uniqueness of these unusual amphipod dynamics it would be extremely useful to perform future studies that could fulfill Koch's postulates. Therefore we would aim to culture the parasite in lab and from that culture re-infect amphipods. Having a cultured parasite would also give us the freedom to study additional factors such as the effect of temperature on pathogenicity. Therefore, future research efforts on this question should focus on cultivating the parasite in the lab and developing protocols for experimental infections with the parasite.

APPENDIX

Figure 8: Saprolegnia PCR gel for replicate 8. DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length. (*) indicates lower DNA concentration.

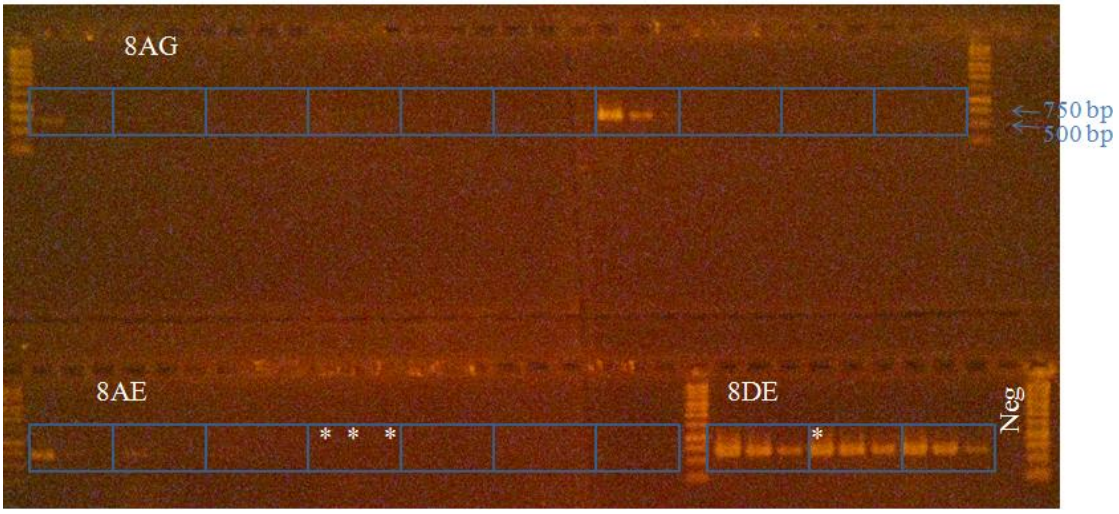


Figure 9: Saprolegnia PCR gel for replicate 7. DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length. (*) indicates lower DNA concentration.

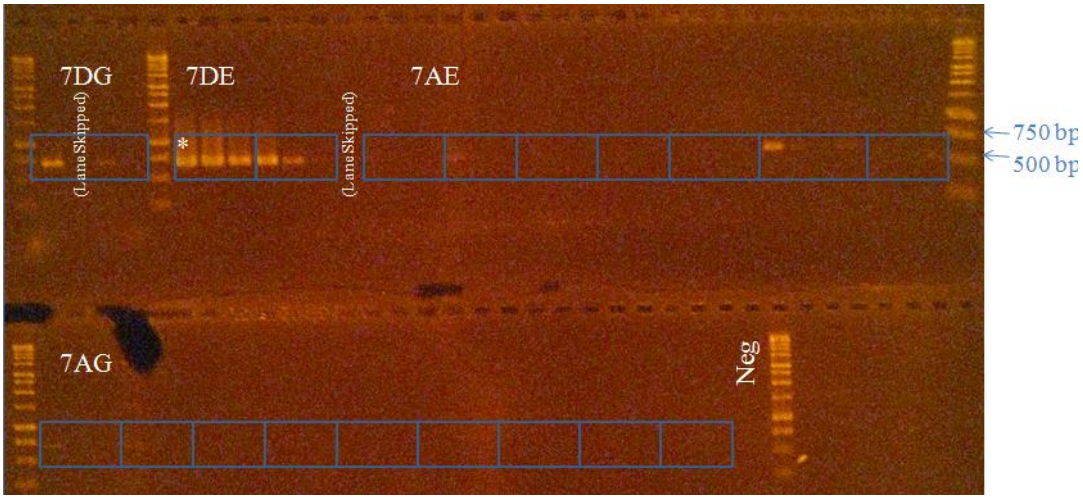


Figure 10: Saprolegnia PCR gel for replicate 5. DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length.

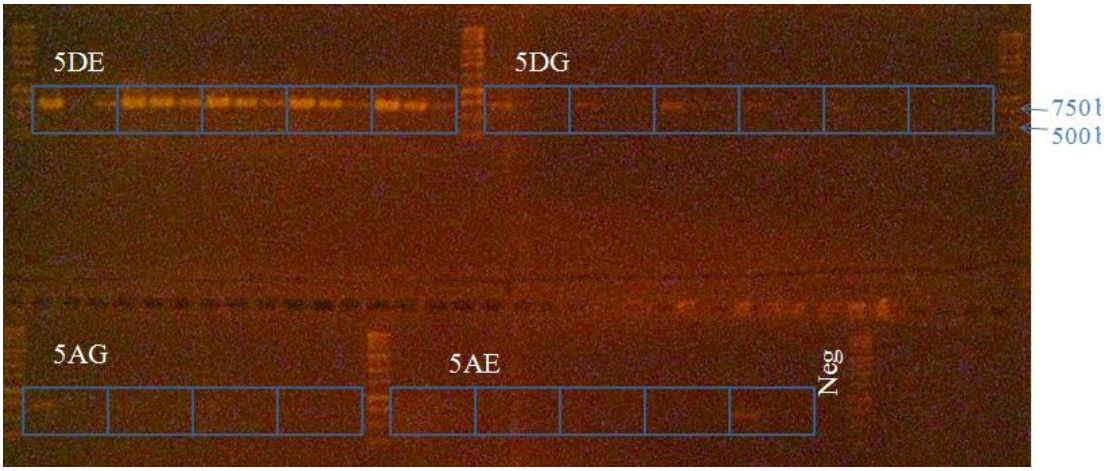


Figure 11: Saprolegnia PCR gel for replicate 10. DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length.

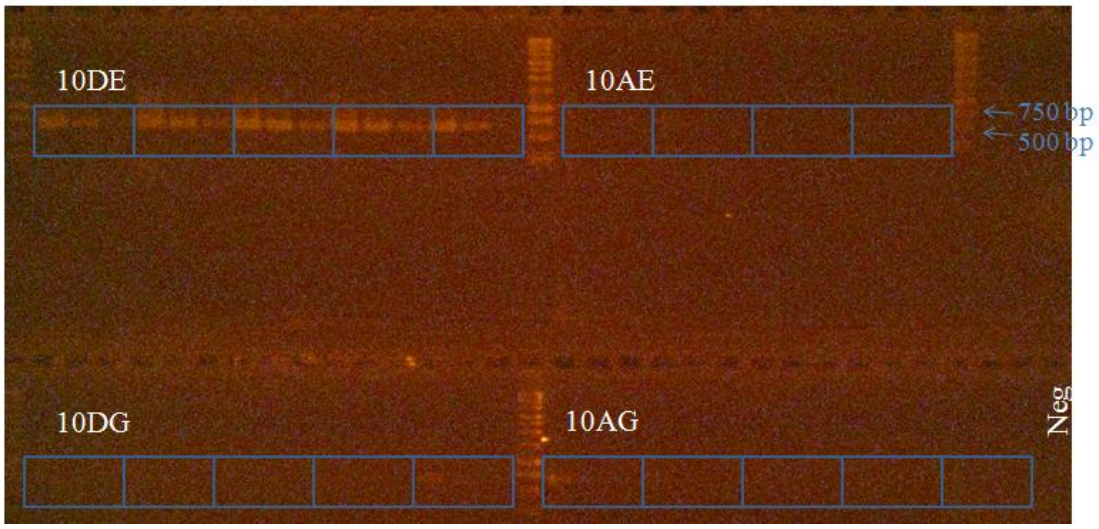


Figure 12: Saprolegnia PCR gel for replicate 14. DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length.

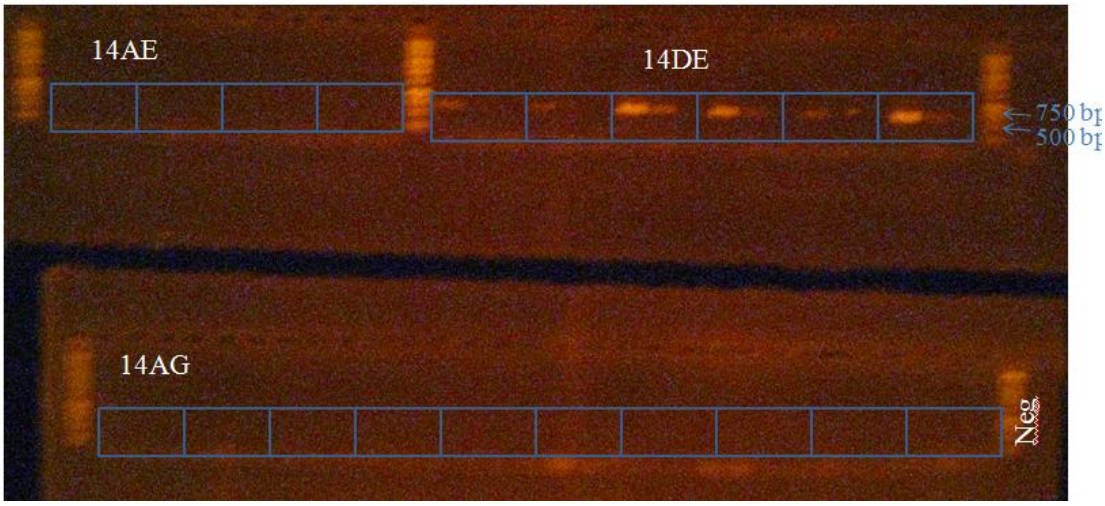


Figure 13: Saprolegnia PCR gel for replicate 1. DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length.

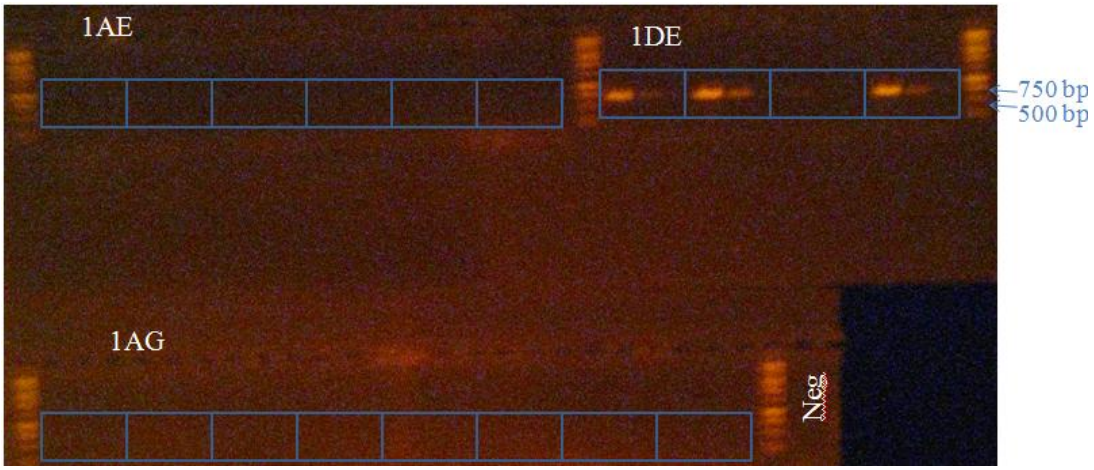
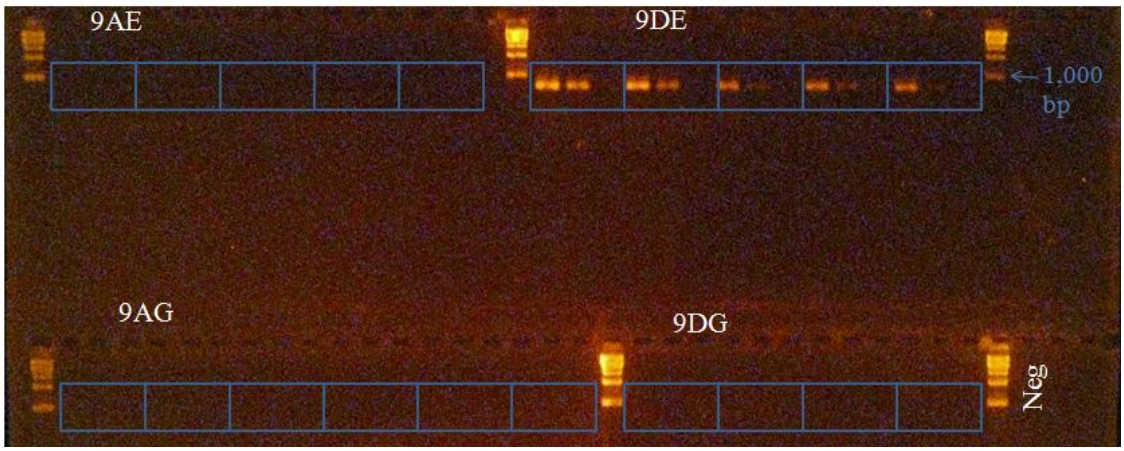


Figure 14: Saprolegnia PCR gel for replicate 9. DNA concentrations of 5 ng, 0.5 ng, and 0.05 ng. The primers bind the 18S rRNA gene at positions 161Fwd and 854Rev; therefore, the amplicon is expected to be 693 base pairs in length.



REFERENCES

Berezina, N. A. (2007). Invasions of alien amphipods (Amphipoda : Gammaridea) in aquatic ecosystems of North-Western Russia: pathways and consequences. [Proceedings Paper]. *Hydrobiologia, 590*, 15-29.

Cornet, S., Sorci, G., & Moret, Y. (2010). Biological invasion and parasitism: invaders do not suffer from physiological alterations of the acanthocephalan Pomphorhynchus laevis. [Article]. *Parasitology, 137*(1), 137-147.

Dick, J. T. A. (2008). Role of behaviour in biological invasions and species distributions; lessons from interactions between the invasive Gammarus pulex and the native G-duebeni (Crustacea : Amphipoda). [Article]. *Contributions to Zoology, 77*(2), 91-98.

Dunn, A. M., & Dick, J. T. A. (1998). Parasitism and epibiosis in native and non-native gammarids in freshwater in Ireland. [Article]. *Ecography, 21*(6), 593-598.

Hay, M. E., Parker, J. D., Deron, E. B., Caudill, C. C., Wilson, A. E., Hallinan, Z. P., et al. (2004). Mutualisms and Aquatic Community Structure: The Enemy of My Enemy Is My Friend. *Annual Review of Ecology, Evolution, and Systematics, 35*(ArticleType: primary_article / Full publication date: 2004 / Copyright © 2004 Annual Reviews), 175-197.

Hirsch, P. E., Nechwatal, J., & Fischer, P. (2008). A previously undescribed set of Saprolegnia spp. in the invasive spiny-cheek crayfish (Orconectes limosus, Rafinesque). [Article]. *Fundamental and Applied Limnology, 172*(2), 161-165.

Hogg, I. D., de Lafontaine, Y., & Eadie, J. M. (2000). Genotypic variation among Gammarus fasciatus (Crustacea : Amphipoda) from the Great Lakes St. Lawrence River: implications for the conservation of widespread freshwater invertebrates. *Canadian Journal of Fisheries and Aquatic Sciences, 57*(9), 1843-1852.

Holdich, D. M., Reeve. (1991). Distribution of freshwater craylish in the British Isles, wiih particular reference

to crayfish plague, alien introductions and water quality. *Aquatic Conservation, 1*(139), 158.

Jacomo , K. P., Raoult D. (2002). Natural history of Bartonella infections. *Clin. Diagn. Lab. Immunol, 9*, 8-18.

Kelly, D. W., Paterson, R. A., Townsend, C. R., Poulin, R., & Tompkins, D. M. (2009). Parasite spillback: A neglected concept in invasion ecology? *Ecology, 90*(8), 2047-2056.

Kiziewicz, B., & Nalepa, T. F. (2008). Some Fungi and Water Molds in Waters of Lake Michigan with Emphasis on Those Associated with the Benthic Amphipod Diporeia spp. [Article]. *Journal of Great Lakes Research, 34*(4), 774-780.

Kohler, S. L., Wiley, M.J. (1997). Pathogen outbreaks reveal large-scale effects of competition in stream communites. *Ecology, 78*, 2164-2176.

MacNeil, C. (2001). Coexistence among native and introduced freshwater amphipods (Crustacea); habitat utilization patterns in littoral habitats. *Archiv Fur Hydrobiologie, 151*(4), 591-607.

Prenter, J., MacNeil, C., Dick, J. T. A., & Dunn, A. M. (2004). Roles of parasites in animal invasions. [Review]. *Trends in Ecology & Evolution, 19*(7), 385-390.

Ramaiah, N. (2006). A review of fungal diseases of algae, marine fishes, shrimps and corals. *Indian J. Mar. Sci, 35*, 350-387.

Settle, W. H., & Wilson, L. T. (1990). Invasion by the Variegated Leafhopper and Biotic Interactions: Parasitism, Competition, and Apparent Competition. *Ecology, 71*(4), 1461-1470.